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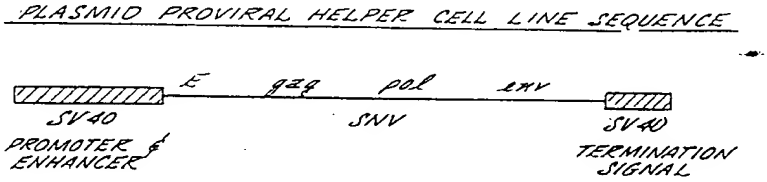

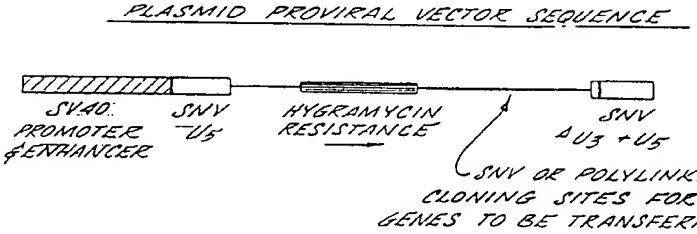

(57) Abstract

A helper cell and helped gene sequence combination for producing replication-defective self-inactivating, retroviral vectors is disclosed. The combination comprises a host cell, a first retrovirus helper gene sequence in the cell, and a second retrovirus helped gene sequence in the cell. The helper gene sequence has a helper region coding for a retrovirus virion protein. The helped gene sequence has a defective 3' U3 region rendering that U3 region incapable of promoting transcription, and further has a defective helped portion which would have rendered the cell unable to form the infectious viral vector which the helped gene sequence codes for if the virion protein had not been supplied from expression of the first retrovirus helper region. Thus, the helper cell produces infectious virions containing a transcript of the second retrovirus helped gene sequence with the virion protein expressed by the first retrovirus helper gene sequence. The defective 3' U3 region is transferred into the 5' end of proviral DNA formed when the infectious virions infect a target cell, rendering the proviral DNA incapable of forming a complete transcript thereof.

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SELF-INACTIVATING, REPLICATION DEFECTIVE, RETROVIRAL VECTORS AND HELPER CELLS FOR PRODUCING THE SAME

Technical Field

This invention generally relates to helper cells useful for producing retroviral gene transfer vectors. More particularly, this invention relates to the production of replication-defective, self-inactivating, retroviral vectors.

Background of the Invention

Retroviruses are viral particles which carry a single strand of RNA, which form DNA from this RNA through the action of reverse transcriptase in a host cell, and which integrate this DNA into the chromosomal DNA of a host cell to form a provirus therein. See Varmus, H., Retroviruses, Science 240, 1427 (1988). Considerable interest has focused on the use of retroviruses as gene transfer vectors. See generally 2 RNA Tumor Viruses, 36-73 (R. Weiss, N. Teich, H. Varmus and J. Coffin 2d ed. 1985). Replication-defective retroviruses are of particular interest as retroviral vectors because they should ordinarily be unable to form new viral particles in a host cell. Such replication-defective retroviruses are, therefore, produced in special helper cells. Id. at 60-61.

U.S. Patent No. 4,650,764 to Temin and Watanabe discloses a helper cell line, useful for producing replication-defective retroviral vectors, which contains a first retrovirus helper gene sequence and a second retrovirus helped gene sequence (the vector coding sequence). The helped gene sequence is expressed by the helper cells as the retroviral vector, while the helper gene

sequence is included to provide one or more viral proteins necessary for the production of a virus particle containing the helped gene sequence and is not itself packaged into a virus particle by the cell. The structural features which achieve these results are a defect in the encapsidation portion of the helper gene sequence and a defect in the protein coding portion of the helped gene sequence. The hypothesis underlying the Temin patent is that, because of the defect in the protein coding portion of the helped (vector) gene sequence, vectors produced by the helper cell are unable to replicate in cells which they infect. The defective encapsidation portion of the helper gene sequence, on the other hand, is intended to preclude RNA transcripts of the helper gene sequence from being themselves packaged into the viral particles.

The helper cell-vector system described by Temin and Watanabe has a number of disadvantages, foremost being the production of significant amounts of replication-competent recombinant virus. See Hu, S. et al., Virology 159, 446 (1987). In reporting on this problem, Hu et al. concluded that "deletion of the packaging sequence alone is not sufficient to prevent viral replication and subsequent genetic recombination." Id. at 449. Hu et al. make no suggestion on how this recombination problem might be solved.

Contamination by replication-competent recombinant virus poses a severe limitation to the use of retroviruses as commercial gene transfer vectors. This is primarily due to the pathogenesis (or potential pathogenesis) of replication-competent virus. In addition, vector sequences containing viral regulatory sequences are undesirable in host cells, as they may cause inappropriate expression of the transferred gene or inappropriate regulation of host genes at the site of insertion. It is also possible that vector sequences that are capable of producing a complete

vector transcript in host cells may recombine with wild-type exogenous virus, which may present biological hazards or other problems.

An object of the present invention is, accordingly, to resolve the recombination problems inherent in the Temin and Watanabe technology discussed above. Other objects and advantages of the present invention are explained in the specification which follows.

Summary of the Invention

A helper cell comprising a host cell, a first retrovirus helper gene sequence in the host cell, and a second retrovirus helped gene sequence in the cell is disclosed. The first retrovirus helper gene sequence has a helper region coding for a retrovirus virion protein. The second retrovirus helped gene sequence has a defective 3' U3 region which renders that U3 region incapable of promoting transcription, and has a defective helped portion which would have rendered the cell unable to form the infectious viral vector which the helped gene sequence codes for if suitable virion protein had not been supplied from expression of the first retrovirus helper region. The helper cell produces infectious virions containing a transcript of the helped gene sequence and incorporating the virion protein expressed by the helper gene sequence. The defective 3' U3 region of the helped gene sequence is transferred to the 5' end of proviral DNA formed when said infectious virions infect a target cell, rendering the proviral DNA incapable of forming a complete transcript thereof in a target cell.

In a preferred embodiment of the present invention, the first retrovirus helper gene sequence has a defective 5' and a defective 3' U3 region rendering both of the U3 regions incapable of promoting transcription, and the second retrovirus helped gene sequence has a defective 5' U3 region

rendering the 5' U3 region incapable of promoting transcription. In this preferred embodiment, the helper cell further comprises a first heterologous promoter operative in the host cell and a second heterologous promoter operative in the host cell. The first heterologous promoter is positioned in the first retrovirus helper gene sequence upstream of the helper region and operatively associated therewith, so that the retrovirus virion protein coded for by the helper gene sequence is produced in the host cell. The second heterologous promoter is positioned in the second retrovirus helped gene sequence upstream of the 5' U5 region of the helped gene sequence and operatively associated therewith, so that a transcript of the second retrovirus helped gene sequence competent to integrate into the genome of a target cell to be transformed is produced in the host cell.

Helper cells of the present invention are used to produce replication defective, self-inactivating, retroviral vectors. Such vectors comprise an infectious virion containing a retrovirus gene sequence. The retrovirus gene sequence has a defective 3' U3 region rendering the U3 region incapable of promoting transcription, and has a defective retrovirus virion protein coding portion which renders the retrovirus gene sequence unable to form the infectious virion in a host cell if suitable virion protein had not been supplied from another source.

In overview, when the helper sequence does not contain any operative U3 regions and the helped sequence does not contain any operative U3 regions, no replication competent retrovirus can be generated by recombinational events in the helper cell or in the target cell. This eliminates the production of replication-competent virus in the helper cell system.

The defective U3 region in the helped gene sequence

advantageously produces a vector that self-inactivates upon entry into the target cell chromosome. The term "self-inactivating" was coined by Yu, S.-F. et al., Self-inactivating retroviral vector designed for transfer of whole gene into mammalian cells, Proc. Natl. Acad. Sci. USA 83, 3194-3198 (1988). Self-inactivation resulting from the inactivation of the 3' U3 region (which would ordinarily serve as the template for the 5' U3 region when the retrovirus integrates as a provirus into a target cell chromosome) eliminates the possibility of the target cell producing a vector RNA transcript; i.e., expression of the complete viral sequence in the target cell following infection with vector virus is blocked. This eliminates the problems of interference of regulation of transferred genes, activation of host genes by inserted vector regulatory sequences, and recombination with wild-type virus. However, in practice this self-inactivation feature is difficult to accomplish with a vector containing an operable 5' U_3 region because, when vector DNA containing an operable 5' U3 region is introduced into the helper cell, recombination occurs between the 5' and 3' LTR, the 3' LTR U3 sequence is regenerated, and the self-inactivating feature is lost. Replacement of the 5' viral regulatory region with a heterologous promoter and enhancer eliminates this problem. However, there is potential for recombination in the helper cells if the viral regulatory region is present. This problem is in turn obviated by rendering the 5' and 3' U3 regions defective in the helper gene sequence and replacing the 5' U3 region of the helper gene sequence with a heterologous promoter. While Yu, S.-F. et al., supra, suggest rendering the 3' U3 region defective in the proviral transcript of a replication competent retroviral vector, they do not suggest incorporating this feature in a replication defective retrovirus, do not suggest rendering

the 5' U3 region of the vector proviral sequence defective to prevent regeneration of the 3' U3 region through recombination, do not suggest deleting the 5' and 3' U3 regions of a helper gene sequence to prevent loss of the self-inactivation feature through recombination in a helper cell, and do not suggest providing a heterologous promoter on a helped gene sequence and on a helper gene sequence once the 5' U3 regions of these sequences have been rendered defective.

The present invention is more fully explained in the drawings, detailed description, and examples which follow. These materials are, however, provided to illustrate various preferred embodiments of the present invention, and are not to be taken as restrictive thereof.

Brief Description of the Drawings

Figure 1 schematically depicts a proviral transcript of a first retrovirus helper gene sequence and a proviral transcript of a second retrovirus helped (or "vector") gene sequence of the present invention.

Figure 2 schematically depicts the construction of pSV_2SNV-2, a helper plasmid carrying a proviral transcript of a first retrovirus helper gene sequence of the present invention;

Figures 3A-3C schematically depict the construction of pVKTR-1, a vector plasmid containing a proviral transcript of a second retrovirus helped gene sequence of the present invention;

Figure 4 schematically depicts the construction of pVKTR-2, an alternative vector plasmid construction to the vector plasmid construction shown in Figures 3A-3C above.

Detailed Description of the Preferred Embodiments

The first retrovirus helper gene sequence and the second retrovirus helped gene sequence of the present invention may be produced from any retrovirus through known

genetic engineering techniques. Retroviruses useful for practicing the present invention include all members of the Retroviridae virus family, encompassing all viruses containing an RNA genome and an RNA-dependent DNA polymerase. See generally 1 RNA Tumor Viruses, 25 et seq. (R. Weiss, N. Teich, H. Varmus and J. Coffin 2d ed. 1984). The genomes of all replication-competent retroviruses contain three genes encoding the virion proteins: gag codes for internal structural proteins, pol codes for reverse transcriptase; env codes for the envelope proteins. Examples of members of the Retroviridae family include fish C-type retroviruses, snake C-type retroviruses, avian retroviruses, murine leukemia viruses, rat C-type retroviruses, tree mouse C-type retroviruses, hamster C-type retroviruses, guinea pig B-type retroviruses, Agonti C-type retroviruses, rabbit C-type particles, feline C-type retroviruses, mink C-type retroviruses, porcine C-type retroviruses, deer C-type retroviruses, horse C-type retroviruses, bovine C-type retroviruses, sheep C-type retroviruses, and primate retroviruses (e.g., prosimian C-type retrovirus, owl monkey C-type retrovirus, Mason-Pfizer monkey D-type retrovirus, Langmur D-type retrovirus). Preferred are the reticuloendotheliosis retroviruses and the murine leukemia viruses. More preferred are the reticuloendotheliosis viruses, including reticuloendotheliosis virus strain T (REV-T), reticuloendotheliosis-associated virus (REV-A), duck infectious anemia virus (DIAV), Trager duck spleen necrosis virus (SNV), and chick syncytial virus (CSV). Most preferred is the SNV. These viruses are set forth for illustrative purposes only, as other retroviruses are known to those skilled in the art. See, e.g., 2 RNA Tumor Viruses, supra at 1 et seq. Those skilled in the art will appreciate that derivatives of retroviruses (e.g., viruses

in which the RNA genome has had segments deleted, segments inserted, or both segments deleted and segments inserted) are useful in the present invention, either directly or after further modification thereof.

The retrovirus from which the first retrovirus helper gene sequence and the second retrovirus helped gene sequence are constructed are selected to produce, in a host cell, a virion which will infect the target cell or cells which it is desired to transform. The term "transform," as used herein, means the insertion of exogenous genetic material into a target cell, preferably into the chromosomal DNA thereof, and not the oncogenic transformation of the target cell into a tumor cell line. This selection can be based on the known patterns of infectivity of the retroviruses. See, e.g., 1 RNA Tumor Viruses, supra. Selection of the host cell can likewise be based on the known host ranges of the retroviruses. Preferred host cells are D17 dog cells, which are available from the American Type Culture Collection in Rockville, Maryland, USA.

The first and second heterologous promoters may be any promoter operable in the host cell, with the heterologous promoter being used in place of the defective 5' U3 promoters so that an operable 3' promoter will not be produced from the 5' promoter through a recombination event and the self-inactivaion feature of the present invention lost. Eukaryotic promoters are usually characterized by two conserved sequences of nucleotides whose locations and structural similarity to prokaryotic promoter sequences (Breathnach & Chambon, Ann. Rev. Biochem. 50, 349-383 (1981)) suggest involvement in the promotion of transcription. The first is a sequence rich in the nucleic acids adenine and thymine (the Goldberg-Hogness, "TATA," or "ATA" box) which is located 20-30 base pairs upstream from the RNA initiation site (the cap site which is the

transcriptional start site for the RNA transcript) and is characterized by a concensus sequence (5'-TATAA-ATA-3'). The second region is the CCAAT box (Efstratiadis, et al., Cell 21, 653-668 (1980)), which is located 70-90 base pairs upstream from the cap site of some genes and has the canonical sequence 5'-GG(C/T)CAATCT-3' (Benoist, et al., Nucleic Acids Res. 8, 127-142 (1980)). The development of techniques for removing and altering these sequences (Nathan and Smith, Ann. Rev. Biochem. 44, 274-293 (1975); Weber, et al., In: D. D. Brown and C. F. Fox (Eds.), Develomental Biology Using Purified Genes, ICN-UCLA Symposium on Molecular and Cellular Biology (New York, Academic Press, 1981), pp. 367-385) has made it possible to separate genes from their promoter regions or portions thereof in order to study their function in heterologous biological systems. Exemplary heterologous promoters for use in practicing the present invention include the SV40 promoter, the bovine papilloma virus promoter, the vaccinia virus promoter, and the mouse metallothionein I gene promoter.

Considerations in designing the defective helped portion of the second retrovirus helped gene sequence, and the corresponding helper portion (coding for a retrovirus virion protein) of the first retrovirus helper gene sequence, are known to those skilled in the art. See, for example, the disclosure of U.S. Patent No. 4,650,764 to Temin and Watanabe, titled "Helper Cell" and issued on 17 March 1987, the disclosure of which is to be incorporated herein by reference.

The second retrovirus helped gene sequence should have an operable encapsidation sequence to facilitate the encapsidation thereof into virion particles. The first retrovirus helper gene sequence may have either a defective encapsidation sequence, as described in U.S. Patent No. 4,650,764 to Temin and Watanabe, or may have an operable

encapsidation sequence. When the first retrovirus helper gene sequence has an operable encapsidation sequence some RNA transcripts thereof will be encapsidated into the infectious virions produced by the helper cell, but when this sequence also has defective 3' and 5' U3 regions it will also be incapable of forming replication-competent virus. Hence, inclusion of the helper gene sequence in the virion vector product of the helper cells of the present invention will have no substantial deleterious effect other than a proportional dilution of the quantity of the second retroviral helped gene sequence therein. Preferably, the LTR regions of the first retroviral helper gene sequence are rendered defective to integrate the helper gene sequence into the chromosomal DNA of target cells. See 2 RNA Tumor Viruses, supra, at 87 (The integrative mechanism appears to act on circular DNA in response to a signal that includes little more than the inverted repeats positioned as they exist in circles with two LTRs.).

The second retrovirus helped gene sequence preferably has inserted therein a heterologous sequence to be integrated into the chromosomal DNA of target cells. Preferably, this heterologous sequence comprises a promoter sequence and a coding sequence positioned downstream of the promoter sequence and operationally associated therewith. The promoter sequence is selected to be operational in the target cell. The coding sequence codes for the production of a protein, or polypeptide fragment thereof, the expression of which is desired in the target cell to be transformed. Accordingly, the heterologous sequence also includes a ribosomal binding sequence operable in the target cell positioned downstream of the promoter sequence, immediately upstream of the coding sequence, and operationally associated with the coding sequence.

Target cells to be transformed by vectors of the

present invention are eukaryotic cells (e.g., fish, reptile, bird, mammal). The cells may be transformed *in vitro* or *in vivo*. An exemplary method of transforming target cells with vectors of the present invention is disclosed in U.S. Patent application Serial No. 07/176,067, filed 31 March 1988, titled "Gene Transfer into Mammalian Animals with Avian Retroviral Vectors," the disclosure of which is to be incorporated herein by reference.

EXAMPLE 1

Construction of Helper Plasmid

A helper plasmid is derived from an SV40 expression vector as shown in Figure 2. The vector pSV_2*cat* is first digested with the restriction enzymes Hind III and Hpa I to remove (deletion = delta) the chloramphenicol acetyltransferase (*cat*) gene. This digestion leaves intact the SV40 promoter and enhancer as well as the polyadenylation signal. An Sst I linker is then inserted in place of the *cat* gene, the new plasmid being called pSV_2delta-*cat*. The *cat* gene is then replaced with the SNV coding region. The plasmid pPUBX (construction described in Example 3 below) containing a deleted proviral copy of the SNV virus is then digested with Cla I and Bam HI to remove most of the remaining viral sequences including the LTR. The new plasmid pPUBXdelta retains .231 Kb of the 5' untranslated sequence. A 6.9 Kb Sst I fragment from pPB101 containing the viral protein coding region is then inserted into the unique Sst I site of pPUBXdelta. This will result in a plasmid containing viral sequences, but without the LTRs. This new plasmid is then digested with Eco RI and Sal I (partial digest) to isolate the fragment containing the viral helper sequences which is then inserted into the pSV_2delta-*cat* plasmid described above. The helper plasmid is called pSV_2SNV-2 and does not contain the retroviral LTR

but does contain the 5' and 3' noncoding sequences including the E region and splice donor. Alternatives include elimination of the E region or constructing two helper cell sequences: e.g., one which contains gag and pol and another which contains env only. This further helps to preclude formation of replication-competent virus.

EXAMPLE 2

Construction of Helper Cells

The transformed dog cell line D17 (obtained from ATCC) is modified such that is contains the helper plasmid pSV_2SNV-2. The resulting cells are called helper cells, as they complement the defect in the defective vectors described in Examples 3 and 4 below. The helper cells produce SNV proteins and package replication-defective SNV (or other REV-derived) vectors.

The helper plasmid pSV_2SNV-2 is introduced (transfected) into D17 cells along with the selectable vector pSV_2neo (containing the neomycin phosphotransferase [neo] gene, which imparts resistance to the antibiotic G418 in cultured cells) to allow selection of genetically transformed cells. Individual colonies of cells are selected under G418 treatment, grown out, and tested to determine if they contain the helper sequences and are capable of producing SNV proteins and packaging SNV virus vectors. Slot blot and Southern blot analysis are used to determine if the modified D17 cells contain the SNV sequences, whereas production of viral proteins is investigated using SDS-polyacrylamide gel electrophoresis and western blot analysis. Packaging function is first tested with previously constructed replication-defective SNV vectors containing the bacterial marker genes cat and neo. Helper cells are then transfected with vector virus DNA and medium collected from the cells is used to infect unmodified

D17 cells and chicken embryo fibroblasts. The presence of CAT and NEO enzyme activity in infected cells indicates that the helper cells are functional.

EXAMPLE 3

Vector Plasmid Construction

The vector is engineered from a proviral copy of the SNV genome contained in the plasmid pPB101 (obtained from ATCC), a pBR322 derivative. The construction requires several steps and is diagrammed in Figure 3. pPB101 is first digested with Xba I and Bgl II (Step 1) to remove most of the viral protein coding region and thus produce a vector that is replication-defective. To facilitate further manipulations, the deleted proviral genome is subcloned into pUC18 by digestion with Eco RI and Sal I (partial) and the new plasmid is called pPUBX. To produce the self-inactivating feature of the vector, the 3' U_3 region is modified to delete the promoter and enhancer but retain the sequences required for integration of the virus into the host genome as well as the termination signal (Step 2). This is accomplished by first subcloning the 3' LTR. pPUBX is digested with Bgl II and Kpn I and the isolated fragment inserted into a modified pUC18 plasmid where the Sst I site has been destroyed. The LTR-containing plasmid, called pPsBK, is then digested with Ava I and Sst I to remove the promoter and enhancer followed by insertion of a Cla I linker and religation to create a unique cloning site in this region for future use. The deleted LTR is then inserted into pPUBX by digestion with Avr II and Kpn I to produce the plasmid pPUBX-LTR. The final modification to the viral sequences occurs at the 5' U_3 region. The retroviral promoter and enhancer are replaced with the SV40 promoter and enhancer (Step 3). pSV_2delta-cat is digested with Sst I and Pvu II to isolate the .340 bp fragment containing the

SV40 promoter and enhancer. This fragment is inserted by blunt end ligation into the plasmid pPsBK that has been digested with Sst I and Ava I to remove the retroviral promoter and enhancer. This new plasmid is first modified to change the Avr II site to a Cla I site and then digested with Cla I and Kpn I to isolate the SV40-LTR hybrid fragment, which is then directionally cloned into a modified pPUBX-LTR plasmid (modification involves digestion with Avr II and Kpn I and blunt ligation to destroy the Kpn I site) at the 5' end of the vector. The self-inactivation feature is then restored by insertion of the Bgl II-Kpn I fragment from pPsBkdelta into the final construct called pVKTR.

To facilitate the production of vector virus in helper cells, a selectable marker gene is added to the vector (Step 4). The hph gene from the plasmid pSV_2hph (obtained from P. Berg) is digested with Hind III and Bgl II to remove the 1.346 Kb fragment containing the hph gene, which is then inserted by blunt end ligation into the Xma III site of the pVKTR (final construct called pVKTR-1). The hph gene is designed to be expressed from the SV40 promoter, therefore expression of this gene is also lost in the host cell due to self-inactivation.

pVKTR-1 has 3 unique cloning sites. The Xba I and Bgl II sites may be used for the insertion of genes to be transferred to host genomes and the Cla I site in the 3' U_3 region may be used to transfer enhancers or other sequences.

EXAMPLE 4

Alternative Vector Plasmid Construction

An alternative vector further eliminates more of the retroviral coding region and adds additional cloning sites for the insertion of transferred genes. The basis for this construction is the KS+ Bluescript vector (obtained from Stratagene Cloning Systems). The SV40-LTR hybrid

fragment isolated by digestion with Cla I and Kpn I is inserted by blunt end ligation at the Sst I site of KS+. The Hind III-Bgl II hph gene fragment is inserted at the Eag I site of KS+. The Avr II-Kpn I fragment containing the deleted LTR is inserted into the Kpn I site. This leaves several additional cloning sites available for the insertion of genes to be transferred. The construction of this alternative vector, pVKTR-2, is shown in Figure 4.

The foregoing Figures, Detailed Description and Examples are illustrative of the present invention, and are not to be taken as restrictive thereof. The scope of the invention is defined by the following claims, with equivalents of the claims to be included therein.

THAT WHICH IS CLAIMED IS:

1. A helper cell and helped gene sequence combination for producing replication defective, self-inactivating, retroviral vectors, comprising:

a host cell;

a first retrovirus helper gene sequence in the cell, said helper gene sequence having a helper region coding for a retrovirus virion protein; and

a second retrovirus helped gene sequence in the cell having a defective 3' U3 region rendering said U3 region incapable of promoting transcription, said helped gene sequence further having a defective helped portion which would have rendered the cell unable to form the infectious viral vector which said helped gene sequence codes for if said virion protein had not been supplied from expression of said first retrovirus helper region;

said helper cell producing infectious virions containing a transcript of said second retrovirus helped gene sequence with the virion protein expressed by said first retrovirus helper gene sequence;

and whereby said defective 3' U3 region is transferred to the 5' end of proviral DNA formed when said infectious virions infect a target cell, rendering said proviral DNA incapable of forming a complete transcript thereof.

2. A helper cell as claimed in Claim 1, wherein said first retrovirus helper gene sequence has a defective 5' and a defective 3' U3 region rendering both of said U3 regions incapable of promoting transcription, and wherein

said second retrovirus helped gene sequence has a defective 5' U3 region rendering said 5' U3 region incapable of promoting transcription, said helper cell further comprising:

a first heterologous promoter operative in said host cell positioned in said first retrovirus helper gene sequence upstream of said helper region and operatively associated therewith so that said retrovirus virion protein is produced in said host cell; and

a second heterologous promoter operative in said host cell positioned in said second retrovirus helped gene sequence upstream of said 5' U5 region of said helped gene sequence and operatively associated therewith so that a transcript of said second retrovirus helped gene sequence competent to integrate into the genome of a cell to be transformed is produced in said host cell.

3. A helper cell as claimed in Claim 2, further comprising a heterologous protein coding region inserted in said second retrovirus helped gene sequence.

4. A helper cell as claimed in Claim 2, wherein said first and second heterologous promoters are SV40 promoters.

5. A helper cell as claimed in Claim 2, further comprising a heterologous termination sequence operative in said host cell positioned in said first retrovirus helper gene sequence downstream of said helper region and operatively associated therewith.

6. A helper cell as claimed in Claim 5, wherein said termination sequence comprises an SV40 termination sequence.

7. A helper cell subcombination useful for producing replication defective, self-inactivating, retroviral vectors, comprising:

a host cell;

a retrovirus helper gene sequence in the cell having a defective 5' and a defective 3' U3 region rendering both of said U3 regions incapable of promoting transcription, said helper gene sequence having a helper region coding for a retrovirus virion protein; and

a heterologous promoter operative in said host cell positioned in said retrovirus helper gene sequence upstream of said helper region and operatively associated therewith so that said retrovirus virion protein is produced in said host cell.

8. A helper cell as claimed in Claim 7, wherein retroviral helper gene sequence is carried by a plasmid.

9. A gene sequence useful in combination with a helper cell for producing replication defective, self-inactivating, retroviral vectors, comprising:

a retroviral helped gene sequence having a defective 5' and a defective 3' U3 region rendering both of said U3 regions incapable of promoting transcription, said helped gene sequence further having a defective helped portion which would render a host cell containing said sequence unable to form the infectious viral vector which the helped gene sequence codes for; and

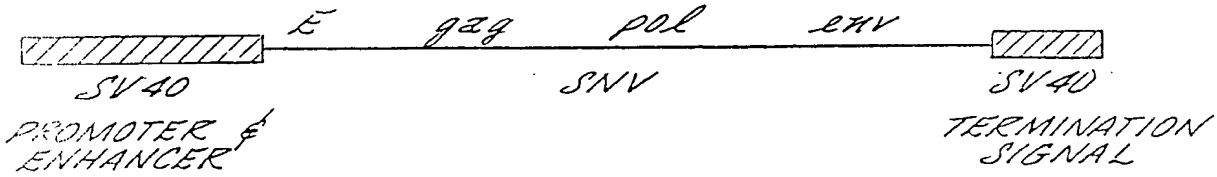
a heterologous promoter positioned in said retroviral helped gene sequence upstream of the 5' U5 region of said second retrovirus helped gene sequence and operatively associated therewith so that a transcript of said second retrovirus helped gene sequence competent to integrate into the genome of a cell to be transformed is produced in a host cell containing said sequence.

10. A gene sequence as claimed in Claim 9, wherein said retroviral helped gene sequence is carried by a plasmid.

11. A replication defective, self-inactivating, infectious retroviral vector, comprising an infectious virion containing a retrovirus gene sequence, said retrovirus gene sequence having a defective 3' U3 region rendering said U3 region incapable of promoting transcription, and having a defective retrovirus virion protein coding portion rendering said retrovirus gene sequence unable to form said infectious virion in a host cell if suitable virion protein had not been supplied from another source.

Fig. 1.

PLASMID PROVIRAL HELPER CELL LINE SEQUENCE



PLASMID PROVIRAL VECTOR SEQUENCE

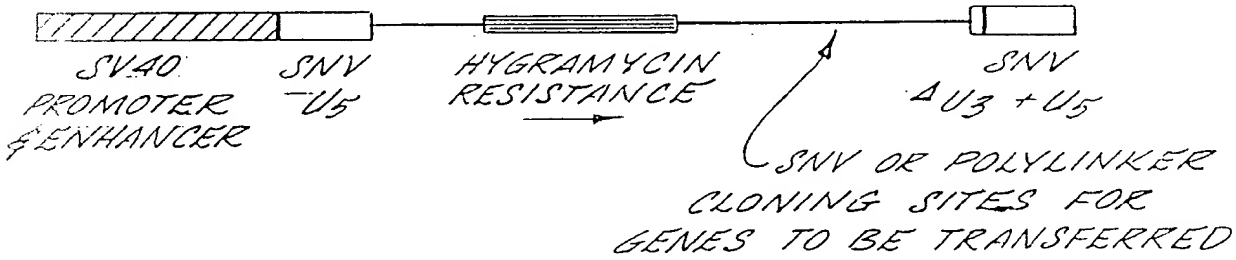


Fig. 2.

HELPER PLASMID CONSTRUCTION

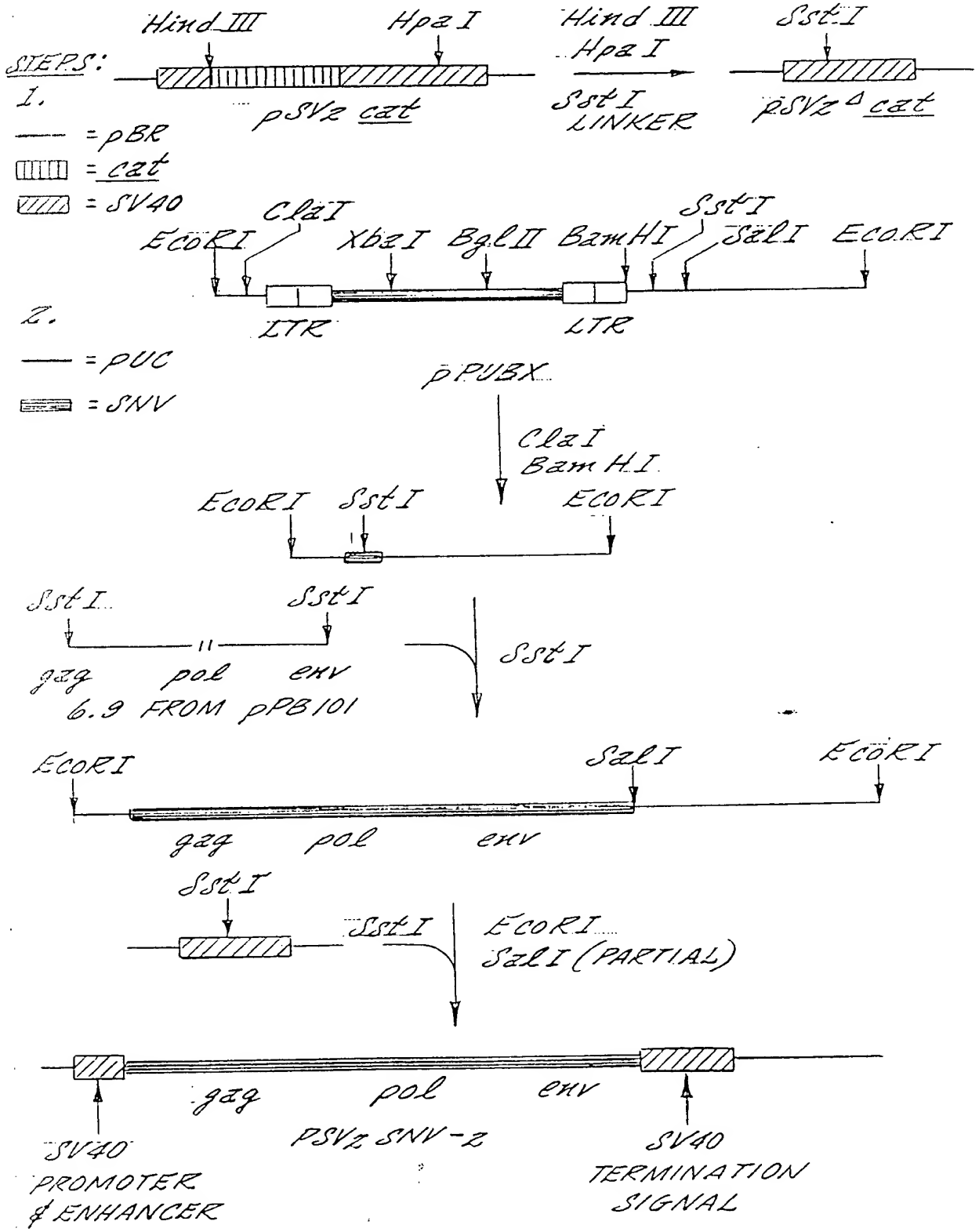


Fig. 3A.

VECTOR CONSTRUCTION

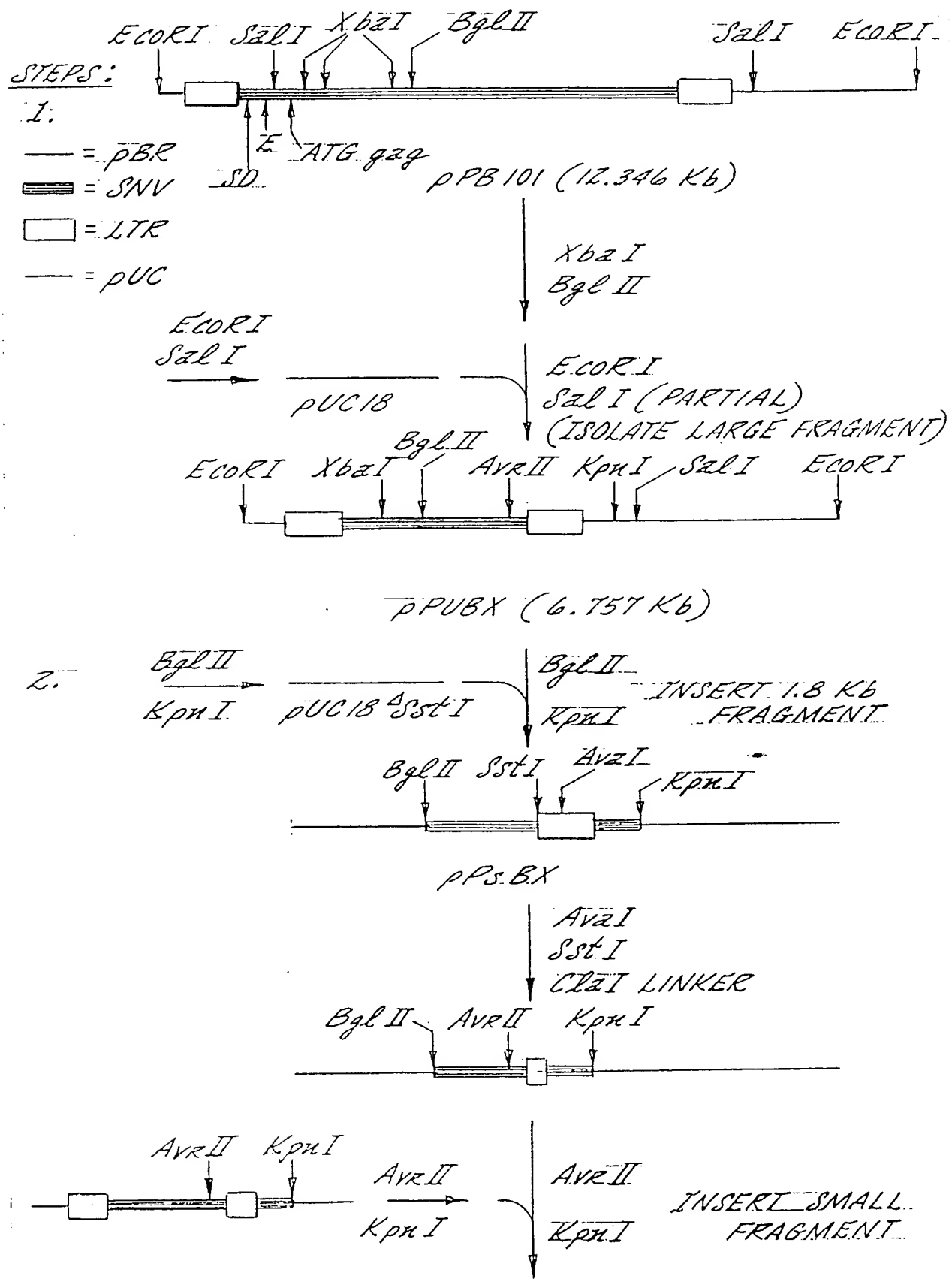


Fig. 3B.

VECTOR CONSTRUCTION CONT.

STEPS:

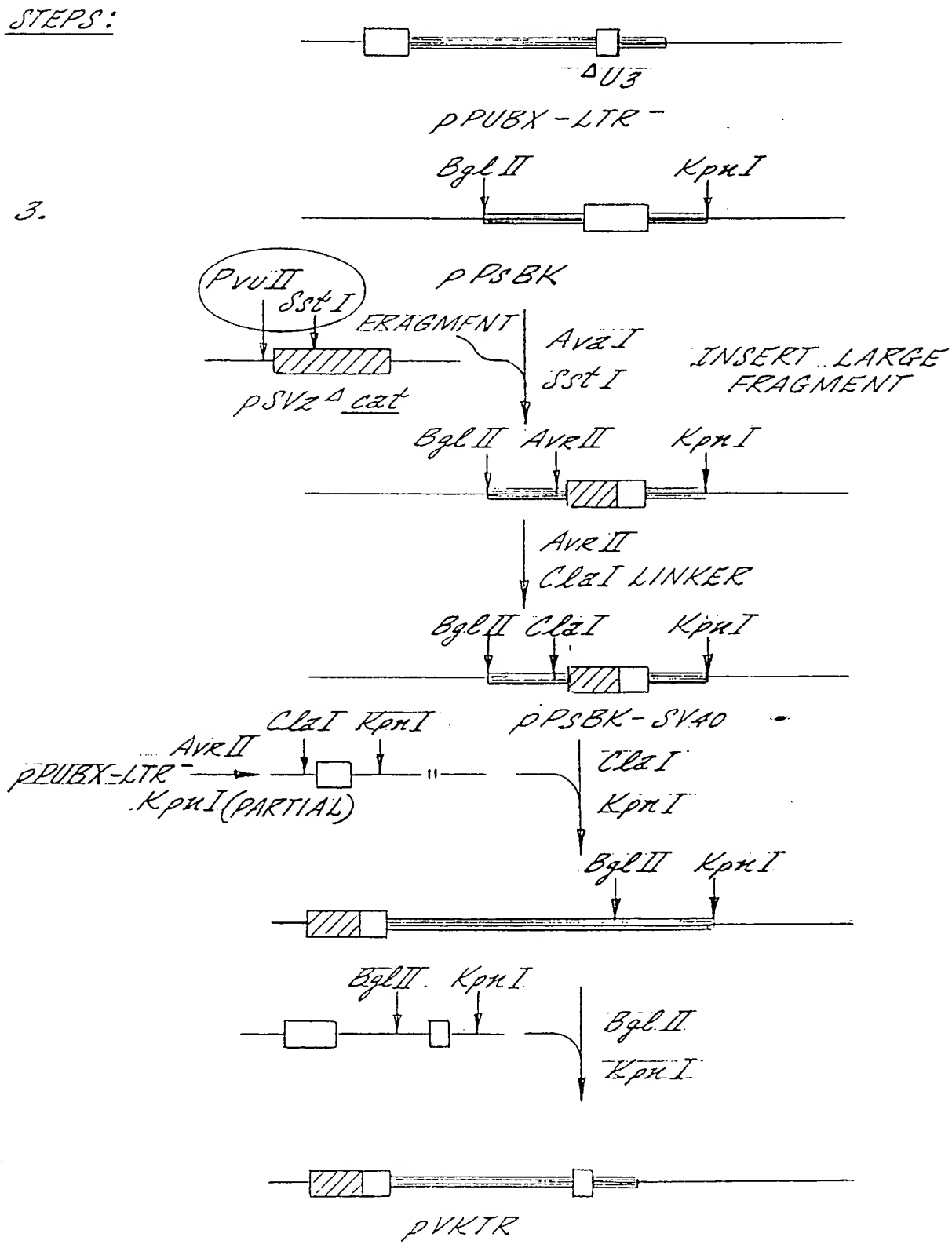


Fig. 3C.

VECTOR CONSTRUCTION CONT.

STEPS:

4.

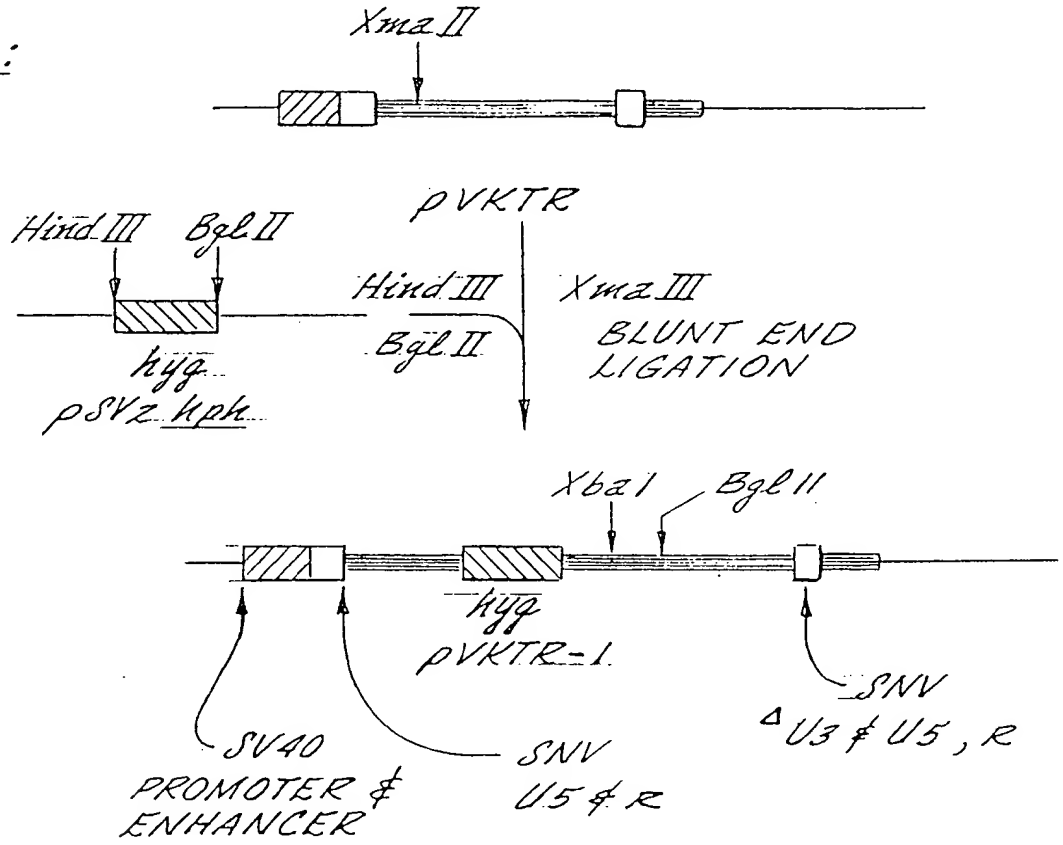
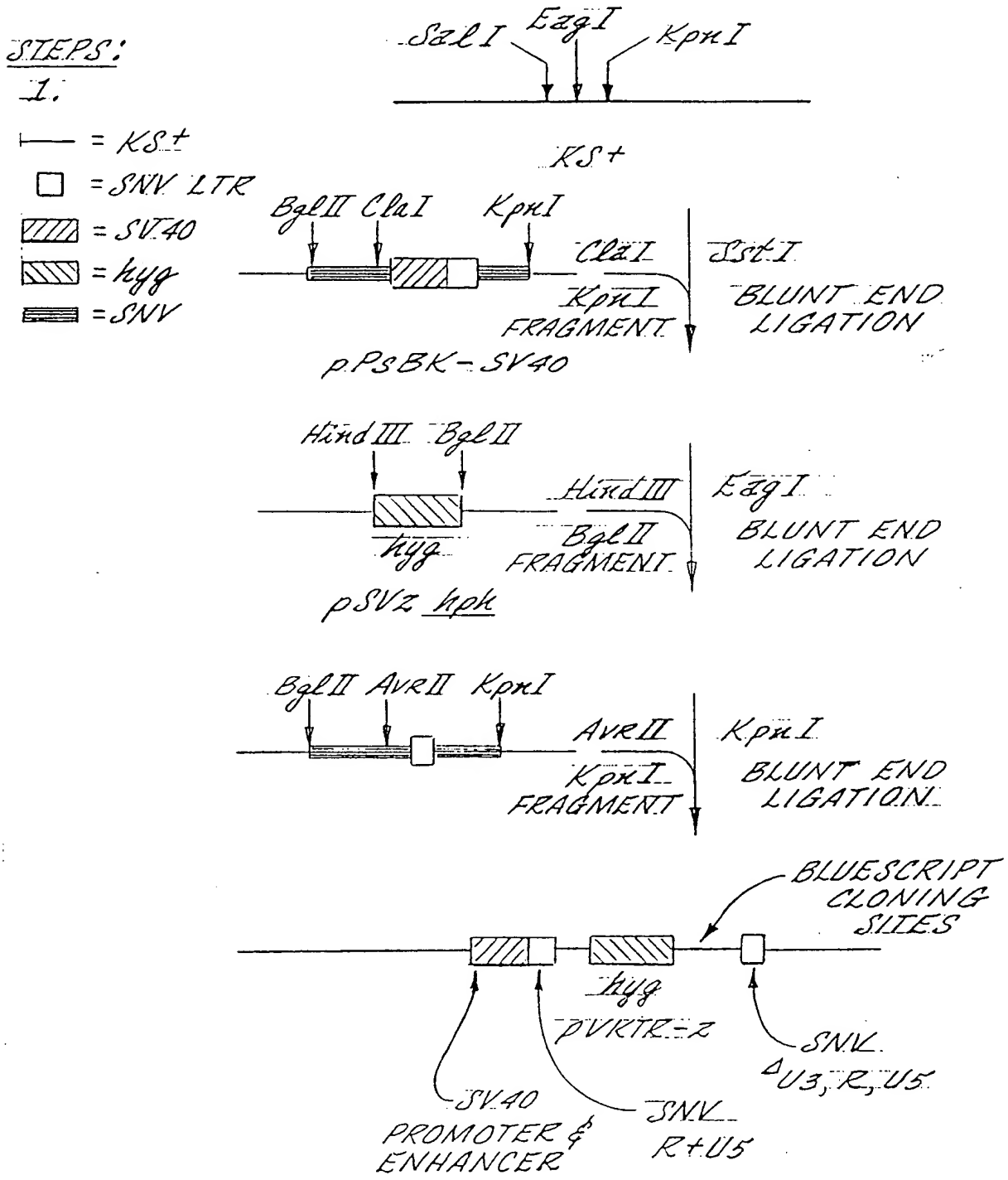


Fig. 4.

ALTERNATIVE VECTOR CONSTRUCTION



INTERNATIONAL SEARCH REPORT

International Application No. PCT/US89/03964

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [6]

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC(4): C12N 5/00, C12N 15/00

U.S. Cl.: 435/172.3, 240.2, 320

II. FIELDS SEARCHED

Minimum Documentation Searched [7]

Classification System	Classification Symbols
U.S.	435/172.3, 240.2, 320, 317.1, 948 536/27 935/32

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched [8]

DATABASES: DIALOG (BIOSIS PREVIEWS, 1969-1989, CA SEARCH, 1977-1989), USPTO AUTOMATED PATENT SYSTEM (FILE USPAT, 1975-19890. SEE ATTACHMENT.

III. DOCUMENTS CONSIDERED TO BE RELEVANT [9]

Category *	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No. [13]
A	US, A, 4,650,764 (TEMIN et al.) 17 March 1987, see the entire document.	1-11
X Y	Proceedings of the National Academy of Sciences, USA (Washington, USA), Volume 83, Issued May 1986, Yu et al., "Self-inactivating retroviral vectors designed for transfer of whole genes into mammalian cells", pages 3194-3198, see the entire document.	11 1-10
Y	Virology (Baltimore, USA), Volume 159, Issued 1987, Hu et al., "Generation of competent virus in the REV helper cell line C3", pages 446-449, see the entire document.	1-11

* Special categories of cited documents: [10]

"A" document defining the general state of the art which is not considered to be of particular relevance

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"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search

06 December 1989

Date of Mailing of this International Search Report

10 JAN 1990

International Searching Authority

Signature of Authorized Officer

Theresa C. Blankenship

INTERNATIONAL SEARCH REPORT

International Application No. PCT/US89/03964

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Signature of Authorized Officer

Jasemine C. Chambers